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Hypomagnetic Field Alters Circadian Rhythm and Increases Algesia in Adult Male Mice*

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Abstract It has been established that exposure in the hypomagnetic field (HMF), which is one of the environmental factor of outer space, has adverse effects on animal and human behavior and brain function. Thus, it is necessary to develop appropriate counteract strategy to avoid the HMF-induced risks to the health of the astronauts during long-term and long-distance manned space mission. However, the physical and mental effects of the HMF in details still await systematic evaluation and the underlying mechanism remains elusive, so far. In this study, we constructed an HMF animal rearing system (< 500 nT) and examined the effects of one-month HMF exposure on the circadian behavior, pain response and emotions in adult male C57BL/6 mice (4～6 weeks old, (20 ± 2) g). The control animals were reared in the geomagnetic field (GMF). The HMF-exposed animals exhibited a prolonged alteration of the circadian drinking rhythm and a decrease in general activity, accompanied with an increase in thermal hyperalgesia. But the HMF did not induce obvious depression-like and anxiety-related behaviors. The serum noradrenalin concentration in HMF-exposed mice significantly decreased. These findings indicate that the HMF disturbs the behavior rhythm and the function of endocrine system, which probably leads to the subsequently weakened activities of the animal.

Key words hypomagnetic field, circadian rhythm, thermal hyperalgesia, emotion, noradrenalin
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Hypomagnetic field (HMF, < 5 000 nT), extremely weaker than the geomagnetic field (GMF, ～50 000 nT), is one of the environmental factors of the outer space[1], such as 220 nT for Martian crust, 1～10 nT for Moon surface and 0.6 nT for inter-planet space. Astronauts will inevitably be exposed to the HMF during long- distance space mission[2]. Recent studies with human subjects showed that short-term HMF-exposure enlarges the pupil size, reduces the vision-related perception capacity[3-4] and also reduces the circulation efficiency[5], suggesting the HMF is a stress factor threating astronauts. Animal experiments showed that the HMF affects behaviors and brain functions. Long-term HMF exposure interrupts the circadian activity of different animals[6-8]. Chicks hatched in the HMF (～2 nT) is impaired in gustation-related memory[9]. Drosophila offspring, bred in the HMF are gradually (10-generations) disabled in vision-related learning and memory[10]. Rat treated in the HMF for 3 months desynchronizes the biorhythms in brain[11]. After long-term HMF treatment (2～6 months), the golden hamster is determined with reduced noradrenalin (NA) level in the brain stem[12]. Short- term or pulsed HMF-exposure reduces stress-induced thermal hyperalgesia in mice[13-15]. HMF treatment (3 hours) lightens seizures of amygdalarly kindled epileptic-model rats[16]. All the evidence indicates adverse effects of the HMF exposure on

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behaviors and brain function of animals and human beings. However, these HMF effects were largely reported sporadically, and conducted in different experimental set ups or on different species [1, 17]. Further investigations are needed to determine whether the effects are specific or consistent in different species, especially in mammals, and to reveal the underlying mechanisms.

For the needs of a comprehensive assessment of HMF effects on adult subjects, especially the astronauts, we extensively studied the behavioral and physiological responses of adult mice to the HMF, including the circadian behavior, pain perception and emotions in an HMF animal rearing system (< 500 nT). Our results showed that 30-day HMF-exposure led to a prolonged alteration of the circadian drinking rhythm on mice, accompanied with an increase in thermal hyperalgesia and a decrease in general activity. But the HMF did not induce significant anxiety and depression-like behaviors. The serum NA level significantly decreased in HMF-exposed mice, while the serum adrenalin level was not changed by the HMF. These findings provide the first systematic assessment of the HMF effects on adult mammal covering the major effective indexes in behavior and physiology, indicating that the HMF environment disturbs the behavior rhythm and NA level of the animal, which probably leads to the subsequently weakened activities.

1 Materials and methods

1.1 Animals and rearing environment

C57BL/6 mice (male, 4 ～6 weeks old) were provided by the animal experiment center of the Institute of Biophysics (IBP), Chinese Academy of Sciences (CAS). Four mice were reared in one standard “shoebox” cage. Food and water were provided *ad libitum* throughout the experiment. Animals were housed under a 12 h/12 h light/dark cycle. The temperature and humidity in the experiment room was maintained at (22 ± 1)℃ and 40%～60%, respectively. After 7 days acclimation in the room, animals were randomly assigned to the GMF or HMF condition and treated for 30 days. All experiments were approved by the Animal Care and Use Committee at the IBP, CAS (authorized No.: SYXK2014-31).

1.2 The HMF condition

A three-axis Helmholtz coils system (HCS, diameter = 2 m) was applied to simulate the HMF for animal rearing. At the center of the HCS, the GMF was compensated to “zero” by a real-time feedback magnetic field control system (Figure 1a). The animal cages of the HMF group were set at places with the residue MF < 500 nT as shown in Figure 1b. The GMF-control animals were reared on a wooden table which is 1.5 m from the HCS in the same room. The static magnetic field (SMF) at the center of the HCS was monitored by a fluxgate magnetometer (National Space Science Center, Beijing, China). The alternating magnetic field (AMF) was measured by a CCG-1000 induction alternative magnetometer (National Institute of Metrology, Beijing, China). The predominant AC field frequency was checked from the output of signal on a Textronics TDS 2014 digital real-time oscilloscope (Tequipment. NET, NJ, USA). The magnetic field conditions are listed in Table 1.

1.3 Behavior tests

Excepting the water drink test, behavior tests were performed from 18 : 00 to 21 : 00. Before the test, animals were allowed to acclimate in the test environments for 2 h. The test arenas and the whole apparatus were cleaned thoroughly by 70 % alcohol to eliminate any odor and trace after each test. The behavior tests, except for the water drink text, were performed in the GMF. To avoid the effect of the GMF exposure during tests, no more than two tests were performed with each animal. Animals performed with NA and adrenalin (AD) assay were no longer applied for behavior assays, and vice versa.

1.3.1 Water drink test

The daily drinking behavior was recorded by cameras fixed at the ceiling (Figure 1a). An animal was considered taking one attempt of drinking when it licks the bottle tip for more than two seconds. The water drinking attempts were counted at 1 h intervals. The counts of total water drinking attempts of one representative cage (four mice with no loss of animals by accident hurt or death) were recorded for each experiment trial.

1.3.2 Tail flick test

Animals were fixed in the test apparatus (Panlab, Barcelona, Spain) and calmed for 20 min. The lower part of the tail was placed at the test point. The tail of the mouse was heat shocked by a laser beam. The latency time of tail flick (T_{tf}) was measured. Each mouse was tested for three times in a 5 min interval per trial.

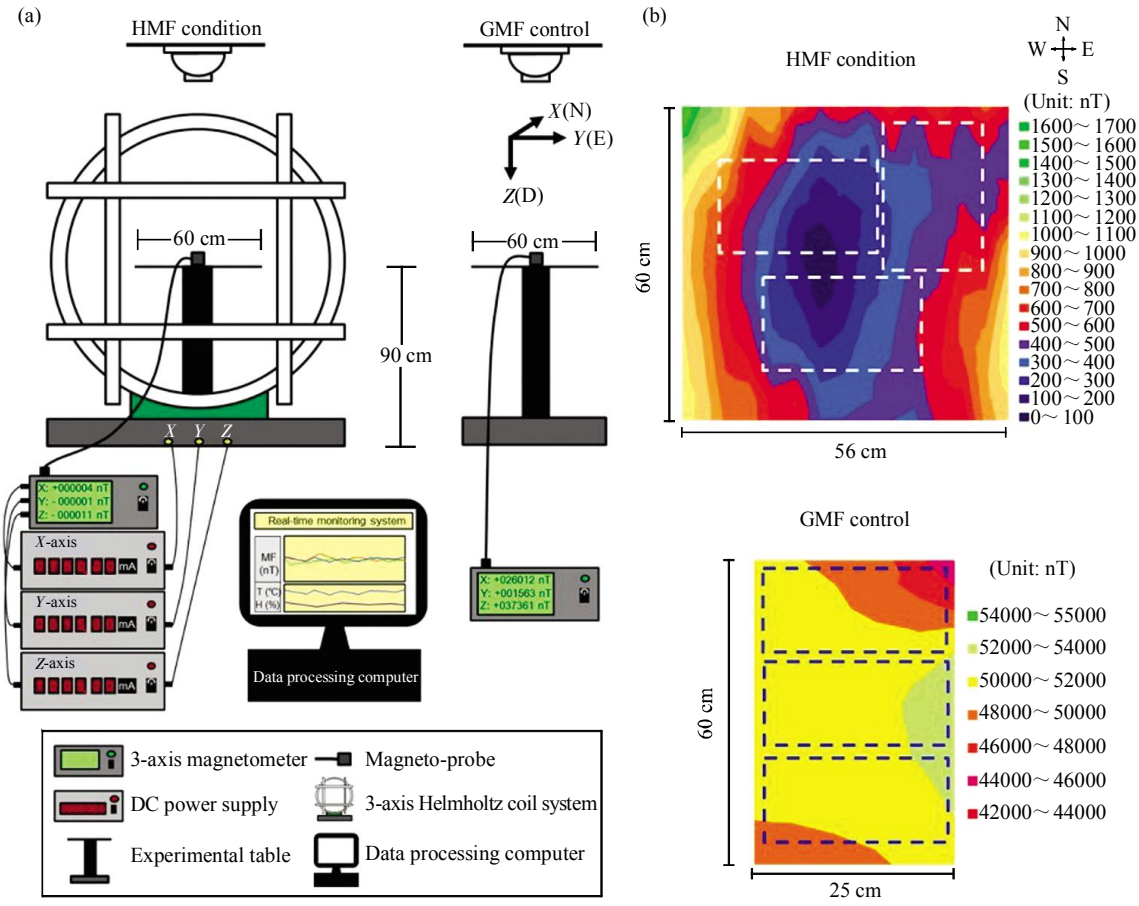

Fig. 1 Experimental set up for animal rearing and magnetic field density

(a) Animals were positioned on the wooden tables centered within the HCS (HMF group) or outside the coils (GMF group). An automatic magnetic field monitoring system compensates the GMF at the center of the HCS to “zero”. Cameras were installed to record the daily activity of the mice. (b) The distributions of the magnetic fields (vector sum) at the central plane of the HCS and the GMF-control table were displayed. The dashed rectangles represent the positions for animal cages.

Table 1 The SMF and AMF conditions [a] (Unit: μT)

	$\|B\|$ [b]	Frequency/Hz	$\|B_x\|$ [c]	$\|B_y\|$ [c]	$\|B_z\|$ [c]
SMF					
HMF_{center} [d]	0.029 ± 0.029	/	0.009 ± 0.018	0.007 ± 0.01	0.023 ± 0.025
$HMF_{avreage}$ [e]	0.55 ± 0.30	/	0.22 ± 0.16	0.37 ± 0.29	0.22 ± 0.22
GMF_{center} [f]	46.3 ± 1.24	/	25.8 ± 1.29	0.85 ± 0.56	38.5 ± 0.64
GMF [g]	42.0 ± 1.10	/	22.5 ± 0.71	0.88 ± 0.69	35.5 ± 0.89
AMF					
HMF_{center} [d]	11.8 ± 1.3	50	/	/	/
GMF_{off} [f]	11.3 ± 0.5	50	/	/	/
GMF [g]	13.6 ± 0.6	50	/	/	/

[a] Data are mean ± SD; [b] Net DC magnetic field (the vector sum); [c] Positive direction of the *X*-axis, South to North; *Y*-axis, East to West; *Z*-axis vertically downward; [d] Residue magnetic field at the center of the HCS; [e] Average magnetic field of the central plane of the HCS; [f] GMF at the center of the HCS with the power supplies off; [g] Average GMF on the control table with the power supplies on.

1.3.3 Forced swim test

A forced swim test system (Panlab) was used to assess the depression level of mice. The cylinders (height = 25 cm; diameter = 10 cm) were filled with 25 ℃ water to a depth of 15 cm. Each mouse was gently placed into a cylinder. Then, the behaviors of mice

were recorded by a top-positioned camera for 6 min. After 2 min habitation, the immobile time (T_i) of each mouse in the following 4 min videos was measured using a Smart software (Panlab). The water was replaced after each test session.

1.3.4 Elevated plus maze test

An elevated plus maze apparatus (Panlab) was used in the experiment. The mice were placed in the central square, their activities were videotaped for 6 min. The last 4 min of the 6 min video was analyzed. A successful entry was counted when four paws of a mouse were all in one arm. The total entries and time spent in the open (E_{open} and T_{open}) and closed (E_{closed} and T_{closed}) arms were measured by a Smart software (Panlab). Total number of arm entries (E_{open} + E_{closed}) and number of closed-arm entries (E_{closed}) reflect general activity level. Open arms time rate [T_{open}/(T_{open} + T_{closed})] and open arms entry rate [E_{open}/ (E_{open} + E_{closed})] reflect anxiety level.

1.4 NA and adrenalin (AD) assay

After the HMF-exposure experiment, animals were anesthetized with diethyl ether and blood samples were collected by eyeball enucleation. Serums were collected by 30 min, 3 500 r/min centrifugation (TDZ5-WS, Xiangyi, Hunan, China) at room temperature. The serum NA and AD levels were determined with ELISA kits (Cusabio, Wuhan, China), according to the operation manuals.

1.5 Statistical analysis

The one-way ANOVA was used for mean comparisons. Chi-square test was used for water drink test. Means were calculated from at least three independent experiments and expressed as mean ± SEM. Differences were significant when $P < 0.05$.

2 Results

2.1 The HMF disturbs the circadian drinking behavior

For animals in the GMF-control group, they displayed a typical rhythmic water drinking behavior: an active drink period from 21:00 to 3:00 (Drink period Ⅰ); a short rest period from 3:00 to 6:00 (Rest period Ⅰ); a short drink period from 6:00 to 10:00 (Drink period Ⅱ); and a long rest period from 10:00 to 21:00 (Rest period Ⅱ) (Figure 2a, d).

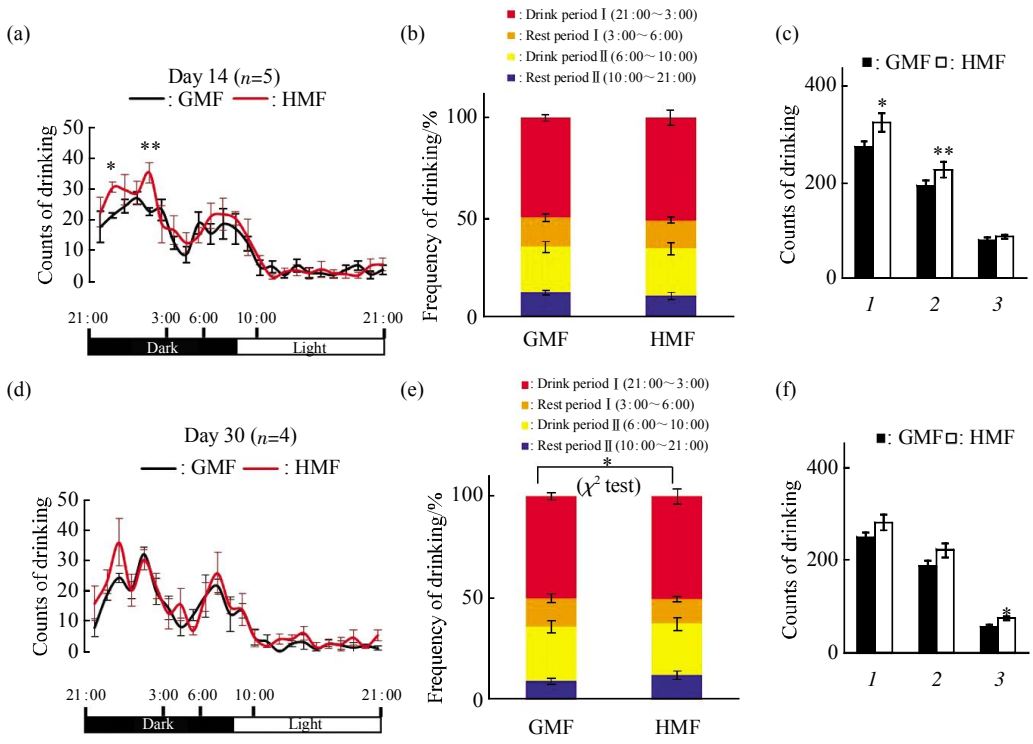

Fig. 2 Water drinking rhythm is altered in the HMF

(a~c) More drinking attempts were recorded in the HMF group at the 22:00~23:00 and 1:00~2:00 intervals. The distributions of water drinking frequencies were the same between the two groups. The total counts of drinking attempts in the HMF were significantly higher than that in the GMF. The counts of drinking attempts at the drink period were increased after 14 days HMF-exposure. (d~f) The drinking attempts at each recording time interval were the same between the two groups, while distributions of water drinking frequencies were significantly different. The drinking attempts were increased significantly at the rest periods. The counts of drinking attempts in different periods were compared by one-way ANOVA. The drinking frequencies were compared with chi-square test. (c, f) *1*: Total; *2*: Drink period(Ⅰ + Ⅱ); *3*: Rest period(Ⅰ + Ⅱ). *n* is the number of experiments. Four animals were used in each group per experiment. Data were shown as mean ± SEM. *$P < 0.05$; **$P < 0.01$.

At day 14, more drinking attempts were recorded in the HMF group at the 22 : 00～23 : 00 (P = 0.010) and 1 : 00～2 : 00 (P = 0.007) intervals (Figure 2a). The distributions of water drinking frequencies within different drinking periods were the same between the two groups. The total drinking attempts in the HMF were significantly higher than that in the GMF (P = 0.019). Especially, the counts of drinking attempts at the drink period rather than the rest period were increased (P = 0.006).

At day 30, the drinking attempts at each recording time interval was the same between the two groups (Figure 2b). However, the distributions of water drinking frequencies were significantly different (P = 0.047). The total drinking attempts in the HMF were the same with the control; while the drinking attempts were significantly increased at the rest periods (P = 0.036). In all, these results showed that the animals became more active after the first 14 days HMF exposure and more disturbed at the rest period after the 30-day HMF exposure.

2.2 The HMF increases thermal hyperalgesia

Tail flick test was conducted to examine the pain response of the mice. A shorter latency time of tail flick (T_{tf}) represents decreased hot pain threshold and increased algesia. After 30-day HMF-exposure, the T_{tf} in the HMF group is significantly smaller than that in the GMF control group (P = 0.0047) (Figure 3a). Therefore, in our experimental condition, the thermal hyperalgesia of adult male mice increases after 30 days in the HMF.

2.3 The HMF does not induce depression and anxiety

Forced swim test was performed to examine the depression-like behavior of the mice. No significant difference between HMF and GMF groups was observed in immobile time (T_i) (P = 0.5725) (Figure 3b), the index of depression, indicating no depression-like behavior was induced by HMF.

The anxiety-related behavior of the mice was examined by elevated plus maze test. The results showed that the HMF significantly reduces the total

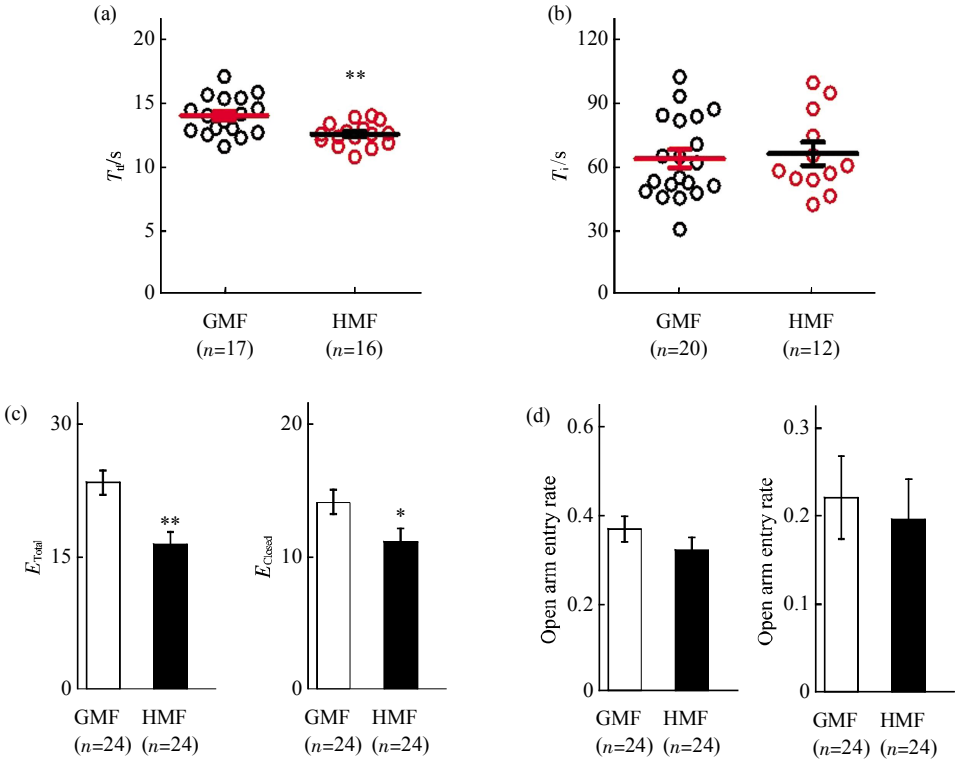

Fig. 3 Thermal hyperalgesia, general activity and emotion-related behaviors

(a) Tail flick test. The HMF-exposed mice showed shorter latency time than the control mice. (b) Forced swim test. The immobility times of the two groups were of no significant difference. (c, d) Elevated plus maze test. The total entry number and closed entry number of the HMF group mice were significantly smaller than those of the controls. The open arm entry rate and open arm time rate did not show notably different levels between the two groups. Data of tail flick test and forced swim test were compared by Mann Whitney test. Data of elevated plus maze test were compared by one-way ANOVA. n is the number of animals from 3 to 6 experiments. Data were shown as mean ± SEM. *P < 0.05; **P < 0.01.

entry number (P = 0.001) and closed arm entry number (P = 0.039), suggesting that the HMF reduces the general activity (Figure 3c). However, the HMF has no effect on anxiety-related behavior in mice (open arm time rate, P = 0.708; open arm entry rate, P = 0.267) (Figure 3d).

2.4 The HMF decreases serum NA level

The endocrine and circulation system was reported to respond to the HMF[5, 15-16, 18]. After 30 days in HMF, serum NA level in mice was significantly lower than the GMF control (P = 0.017), and serum AD level exhibited a trend of decrease but was not significantly different with the controls (P = 0.063) (Figure 4).

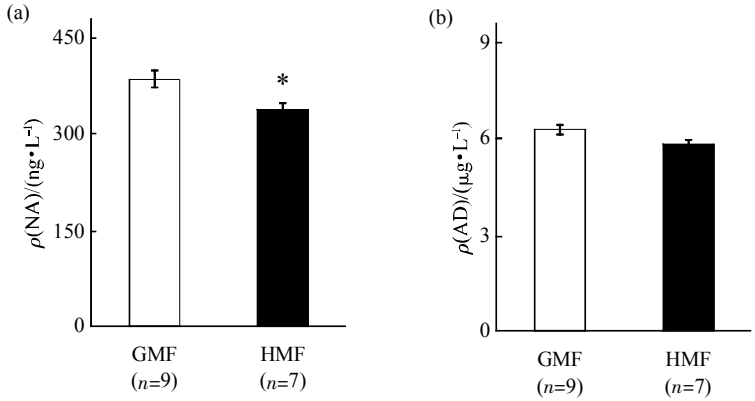

Fig. 4 Serum noradrenaline (NA) and adrenalin (AD) levels

(a) Serum NA level in the HMF-exposed mice was reduced at day 30. (b) Serum AD level is at the same level between the HMF and GMF mice at day 30. Data were compared by one-way ANOVA. n is the number of animals from three experiments. Data were shown as mean ± SEM. *P < 0.05.

3 Discussion

In this study, we systematically assessed the effects of the HMF on behaviors and hormone levels of adult mice under a standardized HMF condition. One-month continuous HMF-exposure notably alters the rhythm of circadian drinking behavior, increases thermal hyperalgesia, and decreases general activity and serum NA level. No significant changes were detected in depression and anxiety-like behaviors in the HMF-exposed mice.

The circadian drinking behavior was changed during the 12 h/12 h day/night circle. Bliss and Heppner showed that birds can use the GMF as a weak Zeitgeber[6]. Rats subjected to 25-day HMF-exposure exhibited increased intraspecific violent at night and decreased food-motivated behavior in the morning[19]. We found that at day 14 of the HMF-exposure, the drinking attempts of adult mice, which largely happened at night-time, increased at the drink period. While at day 30, the drinking attempts increased only at the rest periods (Figure 2). Increased drinking activity at day 14 suggests that the mice consumed more energy during their active period which could contribute to the decrease trend in their body growth[18]. Paradoxical sleep deprivation could reduce the locomotion in rat[20]. The increased drinking activity indicates that mice got less rest in the HMF, which probably leads to decreased general activity in the elevated plus maze test.

It is reported that the short-term HMF-induced analgesia reduction failed to be repeated by an HCS[14], but the effect of long-term HMF-exposure was not tested. Our results demonstrate that long-term HMF-exposure by an HCS reduces the pain threshold to heat, indicating that longer exposure in an HCS could lead to similar effects as short-term exposure in a GMF-shielding box. Additionally, since high-intensity exercise-induced fatigue reduces the neuropathic pain[21], it will be interesting to examine if the reduced general activity we observed also attributes to the increased thermal hyperalgesia in the HMF-exposed mice.

The serum NA level is reduced in the

HMF-exposed mice, and the serum AD also shows a tendency of decrease, indicating the NA system is a target of the HMF. NA is a neurotransmitter, which can be released by both the central and peripheral neurons, and also by adrenal medulla into blood along with AD. NA plays multiple functions, like increasing brain responsiveness and inducing sympathetic responses such as heart rate, trigger the release of glucose from energy stores and increase blood flow to skeletal muscle. The reduction of serum NA level could cause multiple effects related to altered circadian rhythms and decreased general activity, as observed in the HMF. According to previous reports, the decrease in peripheral NA is consistent with the HMF-induced decrease of circulation efficiency[16], and reduction of brain NA levels in HMF-exposed golden hamster and HMF-hatched chicken were accompanied with various behavioral disorders, including inactivation[12, 22]. Exogenous injection of NA could restore impaired long-term memory of chicken[22]. Moreover, both NA and opioid are involved in pathways of anti-nociception, and work as synergic action[23-24]. Prato *et al*. proposed that the HMF effect on thermal hyperalgesia was opioid related, and opioid antagonist could abolished the effect[15], though the possible involvement of NA in this effect was not discussed in their paper. Thus, the down-regulated NA level probably contributes to disrupted behaviors including thermal hyperalgesia in the HMF-exposed animals. In addition, serotonin metabolism has close relationship with circadian rhythm regulation[25], and the HMF can modulate the serum serotonin level[26] and expression of serotonin receptors[27]. Thus, the serotonin system could be another candidate in the further investigation of the mechanism mediating behavioral responses of the mice to the HMF.

In conclusion, our studies give an evaluation of the HMF effects on adult mice covering the major effective indexes in behavior and physiology, and show that the HMF exposure causes a prolonged alteration of circadian drinking rhythms, accompanied with a decrease in general activity, an increase in thermal hyperalgesia, and a reduced serum NA level. These findings provide new cues for further investigating the mechanism of HMF effects on animals, and would benefit developing the health care strategy for the astronauts in the space mission.

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亚磁场引起小鼠昼夜节律改变和热痛觉敏感增加 *

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摘要 地外空间的亚磁场环境是影响宇航员健康的一种潜在风险因素. 动物和人体实验表明，亚磁场显著影响个体行为和神经系统功能. 但是，目前尚缺乏亚磁场对动物行为和生理等多方面影响的系统检测数据. 本文构建了一个适用于动物饲养的亚磁场环境(< 500 nT)，并系统检测了 30 天亚磁场处理对成年雄鼠(C57BL/6， 4～6 周龄，(20 ± 2) g)的昼夜周期、痛觉、情绪及激素水平的影响. 实验结果表明，对比地磁场中饲喂对照组，亚磁场中小鼠的昼夜饮水节律改变、热敏痛觉耐受能力和整体活动水平降低，但是没有发生焦虑或抑郁情绪. 亚磁场处理后，小鼠血清去甲肾上腺素水平显著下降. 这些结果说明一个月连续亚磁场处理扰乱动物的昼夜活动节律和内分泌，随后可能导致其感知觉能力的变化和运动机能的下降.

关键词 亚磁场，昼夜节律，热敏痛觉，情绪，去甲肾上腺素

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